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Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF
CORRECTION UNDER 37 CFR 1.322
Docket No. BKR.110T

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Michel Christian Morre, Brigitte Assouline, Pierre Cortez, Anne Gregoire
Issued : September 8, 2009
Patent No. : 7,585,947
Conf. No. : 9491
For : IL-7 Drug Substance, Composition, Preparation and Uses

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:

Column 11, line 43:

“patents”

Column 14, line 27:

“from GAG and GM.”

Application Reads:

Page 20, line 19:

--patients--

Page 26, line 3:

--from GAG and GAA.--

Column 22, line 30:

“5’ATTCCCATATGGGATTGTGATATTAG
 AAGGTAAAGATGGC3’”

Column 23, line 32:

“BgII”

Column 26, lines 42-45:

“PSIL7EcoRV5’: SEQ ID NO 14
 5’AGATATCATGTTCCATGTTCTTT
 AGGTA3’

EcoRV

PCR products were assayed by agarose gel electrophoresis”

Page 41, line 8:

--5’ATTCCCATATGGGATTGTGATAT
 TGAAGGTAAAGATGGC3’--

Page 43, line 11:

--BgII--

Amendment Under 37 CFR § 1.111 dated November 27, 2007:

--PSIL7EcoRV5’: SEQ ID NO 14
 5’AGATATCATGTTCCATGTTCT
 TTTAGGTA3’

EcoRV

PSIL7MluI3’ SEQ ID NO 15
 5’AACGCGTTCAGTGTCTTTAG
 TGCCCAT3’

MluI

PCR products were assayed by agarose gel electrophoresis--

Column 27, line 45:

“5’TACGCGGCCGCATGTCTCAGAGCAAC
 CGG3’”

Page 51, line 5:

--5’TACGCGGCCGCATGTCTCAGA
 GCAACCGG3’--

Column 27, line 47:

“Bc1XL3’BStBI SEQ ID NO: 15:”

Page 51, line 8:

--Bc1XL3’BstBI SEQ ID NO: 15:--

Column 28, line 38:

“positive done”

Page 52, line 21:

--positive clone--

Column 30, line 45:

“DMA Quantification”

Page 55, line 27:

--DNA Quantification--.

A true and correct copy of pages 20, 26, 41, 43, 51, 52, and 55 of the specification as filed and an Amendment dated November 27, 2007, which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/jb

Attachments: Copy of pages 20, 26, 41, 43, 51, 52, and 55 of the specification
Copy of Amendment dated November 27, 2007

It should be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

5 In the specific case of IL-7 a particular attention should be paid to the immune status of the patient before adjusting dose level. The more the patient is immuno-depressed, as judged for instance through peripheral CD4 T-Cell counts, the less the dose necessary to induce a relative increase in lymphocyte counts. In severely immuno-depressed primates, sub-cutaneous doses of 60 µg/kg daily

10 produce a strong increase in T-Cell counts (X3 to X5, in a dose-dependent manner), but in primates with normal lymphocyte counts, higher doses such as 300 µg/kg are necessary to increase lymphocyte counts from 2 to 5-fold. After sub-cutaneous administration, recombinant IL-7 has a surprisingly long blood half life in primates. Its effect on cell cycling lasts for 24 to 48 hours, allowing efficient treatment

15 schedules with single injection daily down to one injection weekly.

Pharmaceutical compositions according to the invention are preferably administered from once daily down to once weekly, possibly twice weekly, preferably not more frequently than once every 48h, only in order to obtain and/or stimulate patients

20 immune regeneration.

Preferred administration routes are parenteral routes. The parenteral route is preferably an intra-tumoral, more preferably an intra-venous or a sub-cutaneous administration. It includes also intra-arterial, intra-peritoneal or intra-muscular injections.

25 It should be understood, however, that any other suitable administration route may be contemplated depending upon the health status and the reactivity of the patient.

The pharmaceutical composition may comprise additional active ingredients, such

30 as immuno-stimulating agents, preferably selected from a hematopoietic cell growth

been used. The codon encoding Tyr at position 13 (based on the numbering in SEQ ID NO:1) may be selected from TAC or TAT. The codon encoding Glu at position 14 (based on the numbering in SEQ ID NO:1) may be selected from GAG and GAA. The codon encoding Val at position 16 (based on the numbering in SEQ ID NO:1)

5 may be selected from GTT, GTC, GTA and GTG. Finally, the codon encoding Leu at position 17 (based on the numbering in SEQ ID NO:1) may be selected from CTG, CTA, CTT, CTC, TTA and TTG. All combinations of the above indicated codons are possible. A specific example of an altered DS-like sequence corresponds to nucleotides 37-51 of SEQ ID NO:1.

10

A specific, preferred nucleic acid molecule of this invention comprises the sequence of SEQ ID NO:1 and codes for a recombinant human IL-7 polypeptide.

Another specific, preferred embodiment of the invention relates to a nucleic acid

15 molecule as described above which further comprises a signal sequence causing secretion of the produced polypeptide. The signal sequence may be selected from the natural signal sequence of the human IL-7 protein, or from any heterologous signal sequence. Such sequences may originate from other secreted proteins, such as the human growth hormone, or be artificial or synthetic. A preferred signal

20 peptide may be a natural signal peptide of a human growth factor or of a human growth hormone and, more preferably, a natural signal peptide of the human erythropoietin. An even more preferred signal peptide is a synthetic signal peptide such as HMM38. Preferred sequences are those functional in competent mammalian host cells. A specific example of such an improved sequence comprises

25 SEQ ID NO:3, 16 or 18. This sequence comprises a leader sequence and an inactivated SD-like sequence, for improved expression.

Another object of the invention concerns a polypeptide encoded by a nucleic acid sequence as described above, which may be glycosylated or unglycosylated.

30

The human IL-7 encoding cDNA sequence was amplified by polymerase chain reaction (PCR) (Mullis et al.; 1987; Methods in Enzymology; 155:335-350) from human placenta cDNA (BioChain Inc.) using the following specific oligonucleotide
5 primers which contain restriction endonuclease recognition sequences:

- SEQ ID NO: 5: IL75'

5'ATTCCATATGGATTGTGATATTGAAGGTAAAGATGGC3'

Ndel

10

- SEQ ID NO: 6: IL73'

5'AGCCGGATCCTTATCAGTGTTCTTAGTGCCCATCA3'

BamHl

15 The human IL-7-encoding DNA sequence presents, at position 49 after the "ATG" initiation codon, a second putative "ATG" which could behave as a second initiation codon in *E. Coli*, since this second "ATG" is preceded by a "pseudo Shine-Dalgarno" sequence (ribosome binding sequence). To avoid the potential production of an amino-terminal truncated form of r-hIL-7, some of the nucleotides of the "SD-
20 like" sequence were mutated (without modifying the resulting encoded r-hIL-7 amino acid sequence), thereby producing an improved methionyl-IL-7-coding-DNA sequence containing one or more preferred codon(s) for expression in *E. Coli* cells.

25 The suppression of the SD-like sequence in the IL-7-encoding DNA sequence was performed by site directed mutagenesis PCR, using the following oligonucleotide primers:

- SEQ ID NO: 7: mutIL75'

5'TAGGGAATTCCATATGGATTGTGATATTGAAGGTAAAGATGGCAAACAATACGA

30 Ndel

GTCCGTTCTG3'

SEQ ID NO : 9: ptac1

5'ATCGAGATCTAATTCTCATGTTGACAGCTTATCAT3'

BgIII

5

SEQ ID NO : 10 : ptac2

5'ATCGTCTAGGCTGTTCCTGTGTGAAATTGTTATCCG3'

XbaI

- 10 The obtained PCR fragment was loaded on an agarose gel to check for its correct size. It was then digested by the *Bgl*II and *Xba*I and inserted into the pET9a (Novagen) hydrolyzed by the same enzymes. The obtained ligation products were transformed into TOP10 (Invitrogen) competent cells and selected on their kanamycin resistance. The obtained ptac vector was then checked by digestion
15 with several enzymes and by sequencing analysis using oligonucleotides ptac1 and ptac2 as sequencing primers.

The ligation products, containing r-hIL-7 fragment, were transformed into TOP10 competent cells. The selection for plasmid-containing cells was on the basis of the
20 antibiotic (kanamycin) resistance marker gene carried on the vector. Plasmid DNA from a positive clone was isolated from cultured cells, selected by restriction mapping and the correct DNA sequence confirmed by sequencing analysis using pET universal primers as sequencing primers (Novagen) as well as a ptac specific primer:

25

SEQ ID NO : 11 : ptac promoter primer

5'TTCGTGTCGCTCAAGGCCGA3'

The *E. Coli* final expression plasmid comprising SEQ ID NO:1, called ptac-hIL-7 (cf.
30 : Figure n°1), was subcloned into *E. coli* JM101 cells (ATCC).

human Raji lymphoma cells cDNA (Clontech), using the following oligonucleotides as primers.

- SEQ ID NO: 14: BclXL5'NotI

5 5'TAGCGGCCGCATGTCTCAGAGCAACCGG3'

NotI

- SEQ ID NO: 15: BclXL3'BstBI

5'ACTTCGAATCATTCCGACTGAAGAGTG3'

10 BstBI

PCR products were assayed by polyacrylamide or agarose gel electrophoresis in the presence of ethidium bromide and visualization by fluorescence of DNA bands stimulated by UV irradiation. The product band of the size corresponding to the 15 BclXL PCR fragment was isolated and inserted into the plasmid vector pCR II-TOPO (Invitrogen) using the TA-cloning method. The ligation products were transformed into TOP10 competent cells. To select positive clones, plasmid DNA, prepared from cultured individual ampicillin resistant bacterial clones by Plasmid Miniprep Isolation techniques (Biorad), were analyzed, by restriction mapping and 20 confirmed by dideoxy sequencing (Sanger et al.; 1977; Proceedings of the National Academy of Sciences of the USA; 74:5463-5467) of an asymmetric PCR product DNA using pCR II TOPO universal primers as sequencing primers.

Plasmid DNA from a positive clone was digested with restriction endonucleases NotI 25 and BstBI and the resulting fragment was inserted, downstream the pEF_{1α} promotor, into pBudCE4.1-hPSIL-7 vector which had been digested with NotI and BstBI restriction sites.

The resulting mammalian (HEK-293, CHO or BHK) expression vector, comprising 30 SEQ ID NO:3, 16 or 18, is called pBud-hPSIL-7-BclXL (cf.: Figure n°17).

Example B. Fermentation of *E. coli* producing recombinant IL-7

Fermentations for the production of recombinant (human or simian) IL-7 were
5 carried out in 80 liters fermentor (New Brunswick) using an *E. coli* JM101 host strain
transformed with expression plasmid ptac-hIL-7 or ptac-sIL7opt as described in
example A1.

1 L inoculum culture was aseptically transferred into fermentor containing 50 L
batch medium NRJ18 (pH7). The culture was grown in batch mode (T 37°C,
10 agitation: 100-500 rpm D.O₂: 30%). The production phase of the fermentation was
induced by IPTG (200 mg/L) after D.O₂ reached to 0% until the OD-600 of the
culture was about 40. The fermentor content was collected and cooled for at least
30 min in order to reach temperature under 20°C. The culture media was filtered
using a 70 µm filter (PALL R1F-700) to eliminate precipitates and cells were
15 harvested by centrifugation (Beckman J6) at 5000 g for 30 min at 4°C.

For inducible clones, during the 10 days of culture, cells will daily be subtracted from
the perfusion reactor and induced by tetracycline in a batch reactor.

Example C. Fermentation of HEK-293 cells producing recombinant hIL-7

20 The best stable positive clone, as in example A2 was adapted to serum-free
suspension culture by several media screenings in order to produce a clone
optimized for productivity and growth in high cell density culture. Cell culture was
performed in a 3 liters bioreactor with a perfusion system. The cells were allowed to
25 grow to a concentration of 10 millions cells/ml. The reactor was operated at a
continuous perfusion rate of approximately 3 L/day during 10 days. Roughly 30 L of
serum-free culture media containing recombinant protein were generated and used
as starting material for the purification of the r-hIL-7.

- In a Streamline procedure, crude cell culture fluid was transferred directly from the fermentor to the expanded bed of Streamline ion exchange or heparin, , or Sulfopropyl (SP) or Diethyl Aminoethyl (DEAE), followed by a combination of IEX and HIC. Finishing steps may include filtration and concentration. In a traditional
- 5 approach, crude cell culture fluid was clarified using a combination of filtration and concentration [microfiltration (0.45 µm) ultra/diafiltration] steps to isolate the product. The protein solution obtained was loaded onto an ion exchange combination and Heparin Sepharose [Fast Flow (Pharmacia) column] in various combinations to purify the product.
- 10 Finishing steps may also included a Hydrophobic Interaction Exchange (HIC) and a filtration/ultrafiltration (UF) or a Carboxymethyl Ceramic (BioSeptra) purification step for eliminating residual impurities followed by a G25 Sephadex purification step for desalting followed by a Q Sepharose Fast Flow (QFF Pharmacia) which retained various residual contaminants. R-IL-7 drug substance was recovered pure in the
- 15 flow through of the last purification step.

Example F. Product controls and specifications

F1. Drug Substance controls and specifications:

20

CONTROLS	TESTS
IDENTITY	Western Blot Size-exclusion HPLC
PROCESS RELATED IMPURITIES	Escherichia Coli Protein (ECP) Dosage Bacterial Endotoxins Dosage LAL test DNA Quantification (Hybridation, quantitative PCR) SDS-PAGE Silver staining
PRODUCT RELATED IMPURITIES	Reverse phase HPLC Ion Exchange HPLC Size-exclusion HPLC Heparin Affinity HPLC (this control warrant the obtention of the correctly refolded Drug Substance)

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Commissioner for Patents, P.O. Box 1450
Alexandria, VA 22313 on November 27, 2007.

Frank C. Eisenschek
Frank C. Eisenschek, Ph.D., Patent Attorney

AMENDMENT UNDER 37 C.F.R. § 1.111
Patent Application
Docket No. BKR-110T

COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner : Xiaozhen Xie, Ph.D.
Art Unit : 1646
Applicants : Michel Christian Morre, Brigitte Assouline, Pierre Cortez, Anne Gregoire
Serial No. : 10/522,883
Filed : February 2, 2005
Conf. No. : 9491
For : IL-7 Drug Substance, Composition, Preparation and Uses

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 C.F.R. § 1.111

Sir:

In response to the Office Action dated September 4, 2007, please amend the above-identified patent application as follows:

In the Specification

Please replace original Figures 9, 12, and 16 with the attached new Figures 9, 12, and 16.

Please substitute the following paragraph on page 54, beginning at line 12:

In order to eliminate most of the residual impurities including endotoxins, the fraction was adjusted to pH 5 and subjected to a Carboxymethyl Ceramic (BioSeptra) column. The CMC column was equilibrated with loading buffer (50 mM sodium acetate, pH 5). After sample application and washing of the column with washing buffer (50 mM sodium acetate, sodium chloride 0.2 M, pH 6), elution was carried out in one step with buffer (50 mM sodium acetate, 0.8 M sodium chloride, pH 6). Finishing steps may also include a G25 Sephadex purification step for desalting followed by a Q Sepharose Fast Flow (QFF Pharmacia) which retained various residual contaminants. R-IL-7 drug substance was recovered pure in the flow through, as showed shown in figure 9 representing SDS-PAGE analysis: ~~coomassie~~Coomassie blue colored and silver stained.

Please substitute the following paragraph on page 45, beginning at line 14:

Plasmid DNA from a positive clone was digested with restriction endonucleases *Bam*HI and *Nde*I and the resulting fragment, r-sIL-7 encoding DNA sequence, was inserted into ptac vector, as described described in example 1.1., which was also digested with *Bam*HI and *Nde*I restriction sites. The ligation products were transformed into TOP10 competent cells. The selection for plasmid-containing cells was on the basis of the antibiotic (kanamycin) resistance marker gene carried on the vector. Plasmid DNA from a positive clone was isolated from cultured cells, selected by restriction mapping and confirmed by sequencing analysis using T7 terminator universal primer on one hand and ptac promoter primer on the other hand as sequencing primers.

Please substitute the following paragraph on page 49, beginning at line 6 (Applicants have not amended the paragraph; it is being provided to show the text inadvertently covered by the text box of SEQ ID NO: 15):

- SEQ ID NO 14 : PSIL7EcoRV5'

5' AGATATCATGTTCCATGTTCTTTAGGTA3'

EcoRV

- SEQ ID NO 15 : PSIL7MluI3'

5' AACCGCGTTCAGTGTTCTTAGTGCCCCAT3'

MluI

PCR products were assayed by agarose gel electrophoresis in the presence of ethidium bromide and visualization by fluorescence of DNA bands stimulated by UV irradiation. The obtained product band , corresponding to the expected size of the hPSIL-7 cDNA, was isolated and inserted into the plasmid vector pCRII-TOPO (Invitrogen) using the TA-cloning method. The ligation products were transformed into TOP10F' competent cells. To select positive clones, plasmid DNA minipreparations (Biorad), prepared from cultured individual bacterial clones, were analysed by restriction mapping and confirmed by dideoxy sequencing (Sanger et al.; 1977; Proceedings of the National Academy of Sciences of the USA; 74:5463-5467) using pCRII-TOPO universal primers.

In the Claims

1-55. (Canceled)

56. (Currently amended) ~~An IL-7 drug substance comprising, as the active product, an IL-7 A composition of matter comprising a human or simian IL-7 conformer, wherein said conformer comprises the following three disulfide bridges: Cys: 1-4 (Cys2-Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47-Cys141), wherein the total amount by weight of said IL-7 conformer in said drug substance composition of matter is at least 98% by weight and wherein said drug substance composition of matter is substantially free of IL-7 molecular variants or product related impurities.~~

57. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is a recombinant human IL-7 conformer.

58. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 57, wherein said IL-7 conformer comprises the amino acid sequence of SEQ ID NO: 2 or 4.

59. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is a recombinant simian IL-7 conformer.

60. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 59, wherein said IL-7 conformer comprises the amino acid sequence of SEQ ID NO: 12.

61. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is not glycosylated.

62. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is glycosylated.

63. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is associated to the hepatocyte growth factor as a heterodimer.

64. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is functionally attached to a Fc portion of an IgG heavy chain through a peptide hinge region, said IgG being a human IgG1 or IgG4.

65. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein said IL-7 conformer is functionally associated to a Human Serum Albumin (HSA) or a portion of HSA as a fusion protein.

66. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, said drug substance being substantially free of ~~an other~~ another IL-7 conformer.

67. (Currently amended) ~~IL-7 drug substance~~ The composition of matter according to claim 56, wherein the total amount by weight of IL-7 in said drug substance is at least 99.5% by weight.

68. (Currently amended) A pharmaceutical composition comprising an effective amount of a human or simian IL-7 conformer, wherein said conformer comprises the following three disulfide bridges: Cys: 1-4 (Cys2-Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47-Cys141), wherein at least 98% of the total amount by weight of IL-7 consists of said conformer and wherein said composition is substantially free of IL-7 molecular variants or product related impurities ~~a drug substance~~ according to claim 56 and one or more pharmaceutically acceptable carriers.

69. (Currently amended) The pharmaceutical Pharmaceutical composition according to claim 68, wherein the pharmaceutically acceptable carrier is selected from sucrose, trehalose and an amino acid.

70. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 69, wherein the pharmaceutically acceptable carrier is contained in an appropriate buffer to form an isotonic solution.

71. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 70, wherein said appropriate buffer has a pH range comprised of between 5 to 7.5.

72. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 71, wherein said appropriate buffer is an organic salt selected from a sodium citrate buffer and/or an ammonium acetate buffer.

73. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 68, wherein said composition is a lyophilized form.

74. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 68, wherein said composition further comprises a protein or a surfactant.

75. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 68, further comprising an immuno-stimulating agent selected from a hematopoietic cell growth factor, a cytokine, an antigen and an adjuvant, or a combination thereof, for combined, separate or sequential use.

76. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 75, wherein said hematopoietic cell growth factor is selected from the Stem Cell Factor (SCF), particularly the soluble form of the SCF, G-CSF, GM-CSF, Flt-3 ligand, IL-15 and IL-2.

77. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 75, wherein the cytokine is selected from γ interferon, IL-2, IL-12, RANTES, B7-1, MIP-2 and MIP-1 α .

78. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 75, wherein said antigen is selected from a synthetic or natural peptide, a recombinant protein, a killed, inactivated or attenuated pathogen product, a lipid, a portion thereof and a combination thereof.

79. (Currently amended) A pharmaceutical The pharmaceutical composition according to claim 78, wherein said antigen is selected from antigens derived from HIV, Varicella Zoster virus, Influenza virus, Epstein Barr virus, type I or 2 Herpes Simplex virus, human cytomegalovirus, Dengue virus, Hepatite Hepatitis A, B, C or E virus, Syncytium respiratory Respiratory Syncytium virus, human papilloma virus, mycobacterium tuberculosis, Toxoplasma and Chlamydia.

80. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 75, wherein said adjuvant is selected from any substance, mixture, solute or composition facilitating or increasing the immunogenicity of an antigen and able to induce a Th1-type immune response, such as CpG, QS21, ISCOM and monophosphoryl lipid A.

81. (Currently amended) The pharmaceuticalPharmaceutical composition according to claim 68, for administration to a human patient for prophylactic or therapeutic stimulation of B or T lymphocyte development and proliferation, or for enhancement of global or specific immuno-reconstitution, or for enhancement of humoral or cellular immune response.

82. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 68, to prevent or reduce opportunistic infections in immunodeficient patients.

83. (Currently amended) The pharmaceuticalA pharmaceutical composition according to claim 68, to prolong lymphopoiesis stimulation or to produce specific immune response or to broaden the repertoire of a specific immune response in human patients.

84. (Currently amended) The pharmaceutical A pharmaceutical composition according to claim 81, 82 or 83, wherein human patients are immunodeficient patients, cancer patients, patients undergoing grafts, patients infected with a virus or a parasite, elderly patients or any patients having low CD4 count.

85. (Currently amended) The pharmaceutical A pharmaceutical composition according to claim 68, wherein the effective amount of the drug substance is comprised said IL-7 conformer is between about 3 to 300 µg/kg/day, preferably or between 10 to 100 µg/kg/day, and in particular administered from once daily, to twice or three times a week down to once weekly.

86. (Withdrawn) A nucleic acid molecule encoding an IL-7 polypeptide, wherein said nucleic acid molecule comprises an altered Shine-Dalgarno-like sequence.

87. (Withdrawn) A nucleic acid molecule comprising a sequence selected from SEQ ID Nos: 1, 3, 12, 16, 18, 20 or 22.

88. (Withdrawn) A vector comprising a nucleic acid according to claim 86.

89. (Withdrawn) A recombinant host cell comprising a nucleic acid according to claim 87 or a vector containing said nucleic acid.

90. (Withdrawn-currently amended) A The recombinant host cell according to claim 89, wherein said recombinant host cell is a human cell or a bacterial cell.

91. (Withdrawn-currently amended) A The recombinant host cell according to claim 90, which is *Escherichia coli* or *Bacillus Brevis*.

92. (Withdrawn-currently amended) A The recombinant host cell according to claim 90, which is a Chinese Hamster Ovary (CHO), HEK-293 cell line or a human stromal or epithelial cell line.

93. (Withdrawn) An antibody specifically immunoreactive with an IL-7 conformer as defined in claim 56.

94. (Withdrawn) A method of producing an IL-7 drug substance as defined in claim 56, the method comprising:

- a) providing a sample comprising IL-7 polypeptides,
- b) purifying an IL-7 conformer which comprises the following three disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47- Cys141) to produce an IL-7 drug substance, and
- c) optionally, measuring or quantifying, in the drug substance, said particular IL-7 conformer.

95. (Withdrawn-currently amended) The method-of according to claim 94, wherein said sample is obtained from recombinant prokaryotic or eukaryotic host cells producing IL-7 polypeptides.

96. (Withdrawn-currently amended) The method-of according to claim 95, wherein said sample is or derives from a culture of prokaryotic host cells encoding an IL-7 polypeptide and further wherein the method further comprises, prior to step b):

- i) treating said sample to cause a complete denaturation of said IL-7 polypeptides,
- ii) optionally purifying the denatured polypeptide obtained in step i) and
- iii) refolding the polypeptides.

97. (Withdrawn-currently amended) The method-of according to claim 96, wherein step i) comprises the dissolution of inclusion bodies in a denaturant buffer.

98. (Withdrawn-currently amended) The method-of according to claim 96, wherein step ii) is performed by hydrophobic chromatography, ion-exchange or inverse phase chromatography.

99. (Withdrawn-currently amended) The method-of according to claim 97, wherein said hydrophobic chromatography is implemented using HIC butyl.

100. (Withdrawn-currently amended) The method-of according to claim 96, wherein step ii) is carried out at a pH comprised between 6 and 9, preferably between 7 and 8,5 inclusive.

101. (Withdrawn-currently amended) The method-of according to claim 96, wherein said purification step b) comprises the performance of an affinity chromatography.

102. (Withdrawn-currently amended) The method-of according to claim 101, wherein said affinity chromatography is performed on a column of sulfated polysaccharides.

103. (Withdrawn-currently amended) The method-of according to claim 102, wherein the sulfated polysaccharide is dextran sulfate or heparin.

104. (Withdrawn-currently amended) The method-of according to claim 94, wherein the IL-7 conformer is characterized in the drug substance by Mass spectrometry, infra-red spectroscopy, NMR, by determining circular dichroism, by measuring the affinity toward a specific monoclonal antibody raised against said IL-7 conformer, or heparin affinity chromatography, and measured or quantified by ELISA, bioassay or the affinity of said IL-7 conformer for IL-7 receptor and any method of protein quantification if applied to the isolated conformer.

105. (Withdrawn) A method of controlling an IL-7-containing preparation, comprising determining the presence and/or relative quantity, in said preparation, of an IL-7 conformer as defined in claim 56.

106. (Withdrawn) A method of producing an IL-7 drug substance or pharmaceutical composition, said method comprising (i) culturing a recombinant host cell encoding an IL-7 polypeptide, (ii) isolating said recombinant polypeptide to produce an IL-7 drug substance and (iii) optionally, conditioning said IL-7 drug substance to produce a pharmaceutical composition suitable for therapeutic or vaccine use, said method further comprising a step of identifying, characterizing or measuring, in said drug substance or pharmaceutical composition, the quantity and/or quality of an IL-7 conformer as defined in claim 56 and, more preferably, a step of selecting the drug substance or pharmaceutical composition which comprises, as the active ingredient, more than about 98% of said IL-7 conformer.

107. (Withdrawn-currently amended) A The method according to claim 95, wherein IL-7 expression by the recombinant host cells is inducible, regulated or transient, so that the cell culture and IL-7 expression phases can be dissociated.

108. (Withdrawn-currently amended) The method ~~of~~ according to claim 106, wherein the quantity and/or quality of said IL-7 conformer is determined by mass spectrometry-related methods, with or without tryptic digest, circular dichroism, NMR, specific monoclonal antibody analysis for disulfide bridges and/or conformation characterization.

109. (Withdrawn) A method for inducing a prolonged lymphopoiesis stimulation or for amplifying an immune response in a subject, comprising administering to a subject in need thereof an effective amount of an IL-7 drug substance obtained by a method according to claim 94.

110. (Withdrawn) A method for preventing or treating a disease associated with an immunodeficiency, comprising administering to a subject in need thereof an effective amount of an IL-7 drug substance obtained by a method according to claim 94.

111. (New) The composition of matter according to claim 56, wherein said IL-7 conformer is a human IL-7 conformer.

112. (New) The composition of matter according to claim 56, wherein said IL-7 conformer is a simian IL-7 conformer.

113. (New) The pharmaceutical composition according to claim 68, wherein said IL-7 conformer is a human IL-7 conformer.

114. (New) The pharmaceutical composition according to claim 68, wherein said IL-7 conformer is a simian IL-7 conformer.

Remarks

Claims 56-110 are pending in the subject application. Applicants acknowledge that claims 59, 60, and 86-110 have been withdrawn from further consideration as being drawn to a non-elected invention. By this Amendment, Applicants have amended claims 56-85, 90-92, and 95-104, 107, and 108, and added new claims 111-114. Support for the amendments and new claims can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 59-114 are currently before the Examiner with claims 86-110 standing withdrawn from consideration. Favorable consideration of the pending claims is respectfully requested.

The Examiner notes that the listing of references in the specification is not a proper form for an Information Disclosure Statement (IDS). Applicants submitted an Information Disclosure Statement in the subject application on February 2, 2005 and the Examiner has acknowledged consideration of the IDS in the instant Action. Applicants acknowledge that only those references submitted in their IDS filed February 2, 2005 or cited on form PTO-892 have been considered by the Examiner.

The drawings are objected to under 37 CFR 1.83(a) because they fail to show details as described in the specification. Specifically, Figures 9, 12 and 16 are not legible. Applicants have submitted substitute figures for Figures 9, 12, and 16 with this Amendment. Entry and review of the formal drawings is respectfully requested.

Claim 66 is objected to because of informalities. Applicants gratefully acknowledge the Examiner's careful review of the claims. In accordance with the Examiner's suggestion, Applicants have replaced the phrase "an other" with the word "another" in claim 66. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claims 56 and 61-85 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Office Action indicates that the specification does not describe IL-7 conformers, other than human conformers, with disulfide bridges at positions Cys:1-4, 2-5 and 3-6. Applicants respectfully assert that there is adequate written description in the subject specification to

convey to the ordinarily skilled artisan that they had possession of the claimed invention and traverse the rejection.

Applicants note that the Office Action argues that only human forms of IL-7 have the recited disulfide bridges [Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47- Cys141)], citing to Kroemer *et al.* in support of this position (Protein Engineering, 1996, 9(6):493-498). As depicted in Figure 5 of the as-filed application, an alignment of human and simian IL-7 indicates that cysteine residues are found at the positions corresponding to positions 2 and 92, positions 34 and 129 and positions 47 and 141 in both the simian and human polypeptide sequence. Thus, Applicants respectfully submit that both simian and human IL-7 sequences possess cysteine residues at the positions recited within the claims and the as-filed specification provides adequate written description of the claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Claims 71, 80, and 85 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite. The Office Action has rejected the claims for the recitation of “such as” and “comprises”. By way of this amendment, Applicants have removed these terms from the claims and respectfully assert that the amended claims are definite. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claims 56-58, 61, 66, 67, and 81-84 are rejected under 35 U.S.C. § 102(b) as anticipated by Cosenza *et al.* (2000). The Office Action states that Cosenza *et al.* teach a 3-D structure for a recombinant human IL-7 comprising Cys:1-4, 2-5 and 3-6. The Office Action further states that Cosenza *et al.* teach that IL-7 is expressed from *E. coli*, and refolded and purified. In addition, it is indicated that Cosenza *et al.* teach that IL-7 can stimulate pre-B-cell and mature T-cell proliferation, can induce LAK cells and cytolytic T-cells, and may have therapeutic applications in cancer immune therapy and treatment of immune deficiency disease. Applicants respectfully assert that the Cosenza *et al.* reference does not anticipate the claimed invention, particularly, the cited reference fails to teach a drug substance, composition or a composition of matter comprising a human IL-7 or simian IL-7 conformer, wherein said conformer comprises the following three disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47- Cys141), the total amount by weight of IL-7 in said drug substance, composition or a composition of matter is at least 98% by weight and wherein

said drug substance, composition or a composition of matter is substantially free of IL-7 molecular variants or product related impurities.

Applicants submit that the cited reference fails to teach the claimed IL-7 conformers. Contrary to the assertion in the Office Action, Cosenza *et al.* teach that the hIL-7 molecule contains cysteine residues at positions (according to the numbering used in this application) Cys: 1-6 (Cys2-Cys141); 2-5 (Cys34- Cys129) and 3-4 (Cys47- Cys92). This is clearly set forth in the upper panel of Figure 3B within the reference (referring to the Cosenza *et al.* model). This assignment of disulfide bonds is, once again, reiterated at page 918, column 1, *Construction of an IL-7 structural hypothesis*, last sentence where it is stated “The three disulfide bond assignments are composed of cysteine residue pairs (Cys3, Cys142), (Cys35, Cys130) and (Cys48, Cys93)”. These cysteine residues correspond to Cys: 1-6 (Cys2- Cys141); 2-5 (Cys34- Cys129) and 3-4 (Cys47- Cys92) as numbered in this application. Thus, it is clear that Cosenza *et al.* do not teach a human or simian IL-7 conformer containing the following disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34-Cys129) and 3-6 (Cys47- Cys141). It is also noted that the Office Action argues that the 3-D model was constructed for a pure molecule (*e.g.*, IL-7 in a crystal); however it is submitted that a computer generated model is not a drug substance, composition or a composition of matter, rather it is a theoretical representation of a molecule presented in a written form. Furthermore, the model generated by Cosenza *et al.* does not teach the claimed IL-7 conformer. Rather, it teaches a conformer comprising a different set of disulfide bridges (see page 918, column 1, *Construction of an IL-7 structural hypothesis*, last sentence). Thus, it cannot be reasonably asserted that the crystal structure discussed in the Office Action anticipates the claimed invention, particularly in view of the express teachings of the reference with respect to the formed disulfide bonds. Accordingly, the cited reference fails to anticipate the claimed invention and reconsideration and withdrawal of the rejection is respectfully requested.

With respect to a theoretical representation of a molecule only in a written form, the mere written description of a biological material does not normally enable a person skilled in the art to reproduce a specific, claimed biological material. *In re LeGrice*, 301 F.2d 929, 133 U.S.P.Q. 365 (1962)(holding that a mere written description of a “rose floribunda plant” would not normally enable a person skilled in the art to reproduce the plant, since plant breeders “are not presently able

to control the factors which govern the combination of genes and chromosomes required to produce a new plant having certain predetermined desired properties”; that “[s]hould a plant variety become extinct one cannot deliberately produce a duplicate even though its ancestry and the techniques of cross-pollination be known”; and that the prior publication did not meet the legal requirements for the bar stated in 35 U.S.C.A. § 102(b) as it did not communicate where the necessary starting material could be obtained).

Applicants further submit that the holding in *LeGrice* is not limited to plant materials. *Ex parte Argoudelis* (157 U.S.P.Q. 437, 440 (Pat. & Trademark Office Bd. App. 1967), rev'd on other grounds, 58 C.C.P.A. 769, 434 F.2d 1390 (1970)) applied the *LeGrice* holding to claims directed to an isolated antibiotic and methods of making an antibiotic produced by a strain of microorganism and a reference asserted to anticipate the claimed invention. As stated by the Board of Appeals in that decision (regarding the publication cited as prior art), “It cannot be denied that *In re LeGrice* applies to the publication cited in this application to the same extent that it applied to the publications cited in that case. Moreover, we have ourselves held that a written description of the character involved in a case such as the present one is not sufficient to enable a person skilled in the art to produce the invention.” Finally, it is also respectfully submitted that the Court of Appeals for the Federal Circuit has also held that the disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation. *Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research*, 346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003) (holding that “[w]ithout a disclosure enabling one skilled in the art to produce a transgenic mouse without undue experimentation, the reference would not be applicable as prior art). Similarly, chemical compounds that are described in a written form are also not considered to be anticipatory in the absence of an enabling disclosure. See *In re Wiggins*, 488 F.2d 538, 179 U.S.P.Q. 421 (C.C.P.A. 1971)(when a prior art reference merely discloses the structure of the claimed compound, evidence showing that attempts to prepare that compound were unsuccessful before the date of invention will be adequate to show inoperability). In this case, Applicants respectfully submit that attempts to make the claimed compound (human or simian IL-7 conformers having the recited disulfide bonds) were unsuccessful as evidenced by the teachings in the prior art that assigned

disulfide bonds at (Cys3, Cys142), (Cys35, Cys130) and (Cys48, Cys93) (corresponding to cysteine residues Cys: 1-6 (Cys2- Cys141); 2-5 (Cys34- Cys129) and 3-4 (Cys47- Cys92) as numbered in this application). Accordingly, it is respectfully submitted that the cited reference fails to anticipate the claimed reference and reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) is respectfully requested.

Claims 62, 68-80, and 85 are rejected under 35 U.S.C. § 103(a) as obvious over Cosenza *et al.* (2000) in view of Namen *et al.* (U.S. Patent No. 4,965,195), and further in view of Ho *et al.* (U.S. Patent No. 5,714,141). The Office Action indicates that it is obvious to one of ordinary skill in the art to combine the teachings of Cosenza *et al.*, with those of Namen *et al.* and Ho *et al.*, to prepare an IL-7 conformer from a mammalian expression system and use the protein to make pharmaceutical compositions. Applicants respectfully assert that the claimed invention is not obvious over the cited references. As noted above, the IL-7 conformer taught to exist by Cosenza *et al.* contains disulfide bonds at cysteine residue pairs (Cys3, Cys142), (Cys35, Cys130) and (Cys48, Cys93) (page 918, column 1, *Construction of an IL-7 structural hypothesis*, last sentence). These residue pairs correspond to Cys: 1-6 (Cys2- Cys141); 2-5 (Cys34- Cys129) and 3-4 (Cys47- Cys92) as numbered in this application. Thus, it is clear that Cosenza *et al.* do not teach a human or simian IL-7 conformer containing the following disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47- Cys141). Namen *et al.* and Ho *et al.* fail to remedy this defect in Cosenza *et al.* and it is, thus, respectfully submitted that the cited combination of references fails to render the claimed invention *prima facie* obvious. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Claims 63-65 are rejected under 35 U.S.C. § 103(a) as obvious over Cosenza *et al.* (2000) in view of Goldschneiser *et al.* (U.S. Publication No. 2002/0058791 A1) and further in view of Goeddel *et al.* (U.S. Patent No. 5,223,408). The Office Action asserts that Goldschneiser *et al.* teach a hybrid cytokine of IL-7 and HGF β -chain as a pre-pro-B cell growth stimulating factor that exhibit unique lymphopoietic properties and that the IL-7/HGF β can be joined by disulfide-bridges produced by the two polypeptides. The Office Action further asserts that Goeddel *et al.* teach conjugating an immunogenic polypeptide with Ig Fc or albumin to increase half-life. Applicants respectfully assert that the claimed invention is not obvious over this combination of cited references.

As previously argued, the IL-7 conformer taught to exist by Cosenza *et al.* contains disulfide bonds at cysteine residue pairs (Cys3, Cys142), (Cys35, Cys130) and (Cys48, Cys93) (see page 918, column 1, *Construction of an IL-7 structural hypothesis*, last sentence). These residue pairs correspond to Cys: 1-6 (Cys2- Cys141); 2-5 (Cys34- Cys129) and 3-4 (Cys47- Cys92) as numbered in this application. Thus, it is clear that Cosenza *et al.* do not teach a human or simian IL-7 conformer containing the following disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47- Cys141). Goldschneiser *et al.* and Goeddel *et al.* fail to remedy this defect in Cosenza *et al.* and it is respectfully submitted that the cited combination of references fails to render the claimed invention *prima facie* obvious. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Applicants further submit that the claimed IL-7 conformers possess unexpectedly different properties as compared to other forms of IL-7. As is indicated in the specification, purified IL-7 compositions comprising the claimed IL-7 conformers (containing the disulfide bridges: Cys: 1-4 (Cys2-Cys92); 2-5 (Cys34-Cys129); and 3-6 (Cys47-Cys141)) demonstrate biological activities that differ from other IL-7 compositions (see Examples H, I, and J; pages 59-64). These differing biological activities include reduced immunogenicity of the claimed drug substance, composition or composition of matter, increased CD4 T-cell counts in animals treated with the claimed drug substance, composition or composition of matter, and irradiated animals treated with the claimed drug substance, composition or composition of matter exhibited increased CD4 cell counts for a longer period of time as compared to irradiated animals treated with other forms of IL-7 (Figure 13). Accordingly, it is respectfully submitted that drug substances, compositions or compositions of matter containing the claimed IL-7 conformers have unexpectedly different properties as compared to other forms of IL-7 and that these properties are evidence related to the non-obviousness of the claimed invention.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including

any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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FCE/sl

Attachments: Replacement Figures 9, 12, and 16

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,585,947

Page 1 of 3

APPLICATION NO.: 10/522,883

DATED : September 8, 2009

INVENTORS : Michel Christian Morre, Brigitte Assouline, Pierre Cortez, Anne Gregoire

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 11,

Line 43, "patents" should read --patients--.

Column 14,

Line 27, "from GAG and GM." should read --from GAG and GAA--.

Column 22,

Line 30, "5'ATTCCCATATGGATTGTGATATTGAAGGTAAAGATGGC3'" should
read --5'ATTCCCATATGGATTGTGATATTGAAGGTAAAGATGGC3'--.

Column 23,

Line 32, "Bg1II" should read --Bg1II--.

MAILING ADDRESS OF SENDER:

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,585,947

Page 2 of 3

APPLICATION NO.: 10/522,883

DATED : September 8, 2009

INVENTORS : Michel Christian Morre, Brigitte Assouline, Pierre Cortez, Anne Gregoire

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 26,

Lines 42-45,

"PSIL7EcoRV5": SEQ ID NO 14
5'AGATATCATGTTCCATGTTCTTTAGGTA3'

EcoRV

PCR products were assayed by agarose gel electrophoresis" should read

--PSIL7EcoRV5": SEQ ID NO 14
5'AGATATCATGTTCCATGTTCTTTAGGTA3'

EcoRV

PSIL7MluI3" SEQ ID NO 15
5'AACGCGTCAGTGTCTTAGTGCCCCAT3'

MluI

PCR products were assayed by agarose gel electrophoresis--.

Column 27,

Line 45, "5'TAGCGGCCGCATGTCTCAGAGCAACCGG3'" should read
--5'TAGCGGCCGCATGTCTCAGAGCAACCGG3'--.

Line 47, "BclXL3'BstBI SEQ ID NO: 15;" should read
--BclXL3'BstBI SEQ ID NO: 15:--.

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CERTIFICATE OF CORRECTION

PATENT NO. : 7,585,947

Page 3 of 3

APPLICATION NO.: 10/522,883

DATED : September 8, 2009

INVENTORS : Michel Christian Morre, Brigitte Assouline, Pierre Cortez, Anne Gregoire

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 28,

Line 38, "positive done" should read --positive clone--.

Column 30,

Line 45, "DMA Quantification" should read --DNA Quantification--.

MAILING ADDRESS OF SENDER:

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